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Biometrical investigation of water frogs in the Szigetköz Landscape Protection Area

by
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Abstract: An identification function key is given for the discrimination of two water frog forms (*Rana lessonae* – *Rana* kl. *esculenta* population) in the Szigetköz Landscape Protection Area. Distribution pattern of morphometric characters of the two water frog forms represented normal distribution, except inner metatarsal tubercle for both forms and first toe length for female *Rana* kl. *esculenta* specimens. Considerable correlations were found both between body length and tibia length and between tibia length and first toe length for the two water frog forms.
Key words: water frogs, *Rana lessonae* – *esculenta* systems, biochemical analysis, LDH-B isozymes, multivariate analysis, discriminant analysis, morphological analysis

Introduction

Central Europe is inhabited by three distinct forms of water frogs. *Rana ridibunda* Pallas, 1771 and *Rana lessonae* Camerano, 1882 are biologically good species, however, *Rana* kl. *esculenta* Linnaeus, 1758 is a hybridogenetic hybrid between the above two (see Graf & Pelaz 1989 for review). Owing to the hybridization morphological measurements of *Rana* kl. *esculenta* individuals usually show intermediate characteristics between the parent species.

Water frogs were already investigated by Boulenger (1885, 1891) from morphological point of view in the last century (Table 1). Morphological measurements and indices, applied by him constituted the basis of further biometrical studies (Terentjev 1923, 1927). Terentjev (1931) pointed out that strong correlation (0.5-0.9) was found among the morphometric characters and suggested the direct use of the equations of regressions in the systematics of frogs (Terentjev 1936). In most cases, indices computed from measurements have hardly been correlated with each other and/or body length for *Rana ridibunda* (Terentjev 1943).

March (1937) reported a remarkable observation, that the growth rate of extremities, compared to body length has been modified by the maturation, and thereafter a decrease has been found in it. This

Table 1. Range of indexes for three forms of water frogs (Boulenger 1891)

	FTL/IMTL	TL/IMTL
ridibunda	2.5-4.0	9.5-14.0
typica (*esculenta*)	2.0-3.0	7.0-10.0
lessonae	1.5-2.0	5.0 -8.0

FTL/IMTL = first toe length / inner metatarsal tubercle length; TL/IMTL = tibia length / inner metatarsal tubercle length.

above-mentioned phenomenon was also confirmed by Terentjev (1943) and Schuster (1950). Kauri (1954, 1959) considered the water frog forms belonging to one species and investigated their growth patterns of by different geographical regions. Berger (1964, 1966, 1970) queried the conclusions of Kauri (1954, 1959) and found sexual dimorphism and positive correlation between body length and other measurements in the cases of adult water frogs. The variability of morphometric indices (FTL/IMTL, TL/IMTL, BL/TL) by developmental stage has also been demonstrated. Juszczyk (1971) studied the morphometric structure of green frog populations on a large scale and confirmed the appearance of three distinct forms of green frogs.

The allometric growth rate of morphometric characters was also studied in *Rana ridibunda* (Opatrny 1966). Günther (1968, 1973) investigated biometrical ratios of BL/TL, TL/IMTL and FTL/IMTL, respectively. According to Wijnands & Van Gelder (1976), two indices (BL/IMTL and BL/IMTW*) were found to be the most important discriminating features between the three species.

Gubányi (1992) and Gubányi & Korsós (1992) published further results on multivariate analyses of selected morphological measurements based on biochemical identification of the species, and claimed the importance of two ratios BL/FTL and TL/IMTL, which were not sexually dimorphic, as possible key characters for determination of the diploid water frog forms. At the same time, the population specific variability of morphometric characters was demonstrated for diploid and triploid *Rana* kl. *esculenta* specimens (see Plötner et al. 1994 for review).

The Department of Zoology of the Hungarian Natural History Museum has undertaken a long term monitoring program of animal and plant communities, including amphibians (see Gubányi & Creemers 1994), in the Szigetköz, situated on the floodplain of the River Danube. The aim of the study was to examine the morphometric characters of a mixed *Rana lessonae* – *Rana esculenta* population system and provide a practical identification function key for diploid *Rana lessonae* and *Rana* kl. *esculenta* individuals in the inland delta of the River Danube. Water frog populations were also studied in the Slovakian part of the floodplain of the River Danube (Csallóköz) by Lác (1959) and Kux (1975).

Materials and methods

The study was carried out between 1989-1993. The frogs were collected at the following localities:

1. Cikolasziget (Győr-Sopron-Moson county), a temporary pond (about 200 m^2), surrounded with poplar (*Populus canadensis*) in the floodplain along the dam.
2. Ásványráró (Győr-Sopron-Moson county), temporary ponds and dead arms and channels in the floodplain, close to the dam, covered by *Rorippa amphibia*.
3. Lipót (Győr-Sopron-Moson county): shallow, gravelled permanent channels without submerged aquatic plant vegetation in the floodplain; and Bár-Duna ér (a channel) near the village.
4. Hédervár (Győr-Sopron-Moson county), 50 m long stretch of Lipót-Hédervári channel, covered by *Ceratophyllum demersum* in the vicinity of the village.
5. Patkányos (Győr-Sopron-Moson county), parts of the dead arms of River Danube in the floodplain.
6. Nagybajcs: (Győr-Sopron-Moson county): temporary waters in the floodplain.

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* BL = body length, TB = tibia length, FTL = first toe length, IMTL = inner metatarsal tubercle length, IMTW = inner metatarsal tubercle width

For biometrical analysis 1326 frogs were tested. Measurements were taken with a vernier calliper on the left side of the living animals. Four morphometric characters were measured: BL – body length; TB – tibia length; FTL – first toe length; IMTL – inner metatarsal tubercle length. From the above measurements the following two additional ratios were computed: BL/FTL and TL/IMTL.

In the beginning of the study identification and ploidy level of water frog forms were based on electrophoretic phenotype of LDH-B isozyme. Afterwards an identification function key was computed based on the comparison of morphometric indices and electrophoretic phenotypes of LDH isozyme (see Gubányi 1992 for review).

Blood samples were taken from the hind limb of the frogs using a micropipette and diluted with amphibian Ringer-solution. Finally, samples were centrifuged (5 min, 4500 rpm) and stored at 25 °C until processing. Lactate dehydrogenase (LDH, EC 1.1.1.27) was studied by the method of vertical polyacrilamid gel electrophoresis after Maurer (1971). The isozymes were localized by a standard procedure (cf. Gubányi & Pekli 1991).

Calculations of descriptive statistics, significance of differences (Mann-Whitney U-test), frequency distributions, allometric properties, correlations and discriminant analysis were performed by using SPSS/PC + programme package (Norusis 1990).

Results

After comparing the electrophoretic phenotypes of LDH-B isozyme and morphometrical indices by using the Fisher's linear discriminant function, the following identification function key was calculated for the two water frog forms:

Rana lessonae	0.094LC/DP + 2.866 LT/CIL < 20.44*
Rana kl. *esculenta*	0.094LC/DP + 2.866 LT/CIL ≥ 20.44

As shown in Table 2 applying discriminant analysis to LC/DP and LT/CIL indices gave correct classification percentage of the two water frog forms. The classification result of the discriminant analysis was correct at a high level for *Rana lessonae* specimens (more than 90

Table 2. Classification results on the basis of discriminant analysis applied to ratios body length / first toe length and tibia length / inner metatarsal tubercle length (actual groups were identified by the means of electrophoretic phenotypes of LDH-B isozyme, percentage is given in parenthesis)

	Predicted group	
Actual group	*R. lessonae*	*R. esculenta*
Rana lessonae	67 (91,8)	6 (8.2)
Rana esculenta	11 (22.9)	37 (77.1)
Percent of frogs correctly classified (%)		85.95

* values of indices are in mm

Table 3. Descriptive statistics for the water frog forms

	Rana lessonae							
	male (n = 357)				female (n = 237)			
	BL	TL	FTL	IMTL	BL	TL	FTL	IMTL
Mean	52.19	23.46	6.29	3.72	54.45	24.05	6.46	3.80
Std.dev.	4.70	2.19	0.75	0.41	7.17	3.08	0.99	0.52
Min.	41.00	18.40	3.40	2.80	41.00	17.70	4.30	2.80
Max.	71.00	30.40	8.70	5.00	78.00	37.60	9.60	5.50
	Rana kl. *esculenta*							
	male (n = 212)				female (n = 520)			
	BL	TL	FTL	IMTL	BL	TL	FTL	IMTL
Mean	53.51	24.91	6.60	3.35	61.33	29.19	7.84	3.71
Std.dev.	6.18	3.74	1.10	0.41	11.72	5.84	1.70	0.71
Min.	43.00	18.30	3.00	2.50	40.00	17.10	4.20	2.20
Max.	77.00	40.10	11.20	5.00	100.00	46.90	13.40	6.00

Table 4. Results of the multiple regression analysis of linear measurements for the water frog forms

	Rana lessonae							
	Dependent variables							
	Males				Females			
Indep. v.	BL	TL	DP	CIL	BL	TL	DP	IL
BL	–	0.52	0.26	0.17	–	0.42	0.20	0.30
TL	0.69	–	0.74	0.77	0.70	–	0.98	0.74
DP	0.08	0.17	–	0.03	0.09	0.27	–	-0.08
CIL	0.07	0.26	0.05	–	0.16	0.24	-0.09	–
Multip.R.	0.89	0.92	0.79	0.82	0.94	0.96	0.90	0.89
R Square	0.79	0.85	0.62	0.67	0.88	0.92	0.81	0.79
	Rana kl. *esculenta*							
	Dependent variables							
	Males				Females			
Indep. v.	BL	TL	DP	CIL	BL	TL	DP	CIL
BL	–	0.76	0.39	0.44	–	0.52	0.07	0.66
TL	0.52	–	0.66	0.36	0.65	–	0.96	0.25
DP	0.09	0.19	–	0.01	0.03	0.41	–	0.02
CIL	0.19	0.22	0.02	–	0.24	0.07	0.01	–
Multip.R.	0.94	0.94	0.85	0.86	0.97	0.98	0.95	0.91
R Square	0.89	0.89	0.72	0.74	0.94	0.96	0.91	0.84

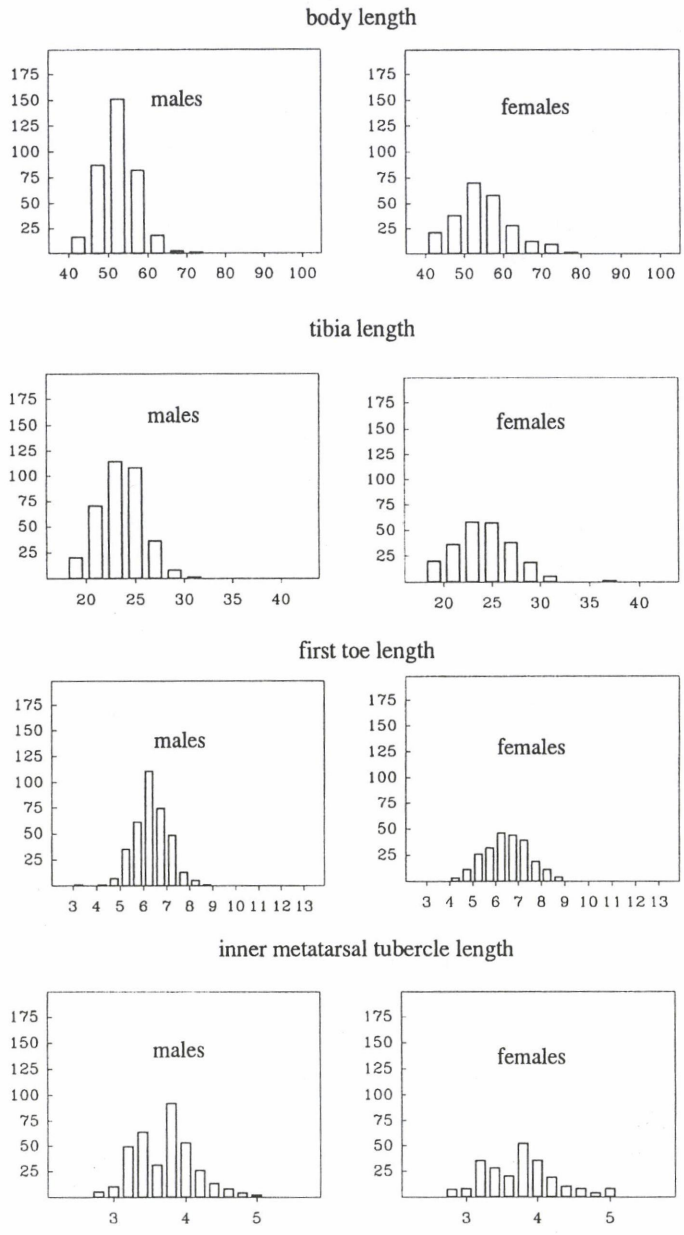

Fig. 1. Frequency distribution of morphometric characters for *Rana lessonae* (horizontal axes represent the measurements in mm; vertical axes represent the number of specimens)

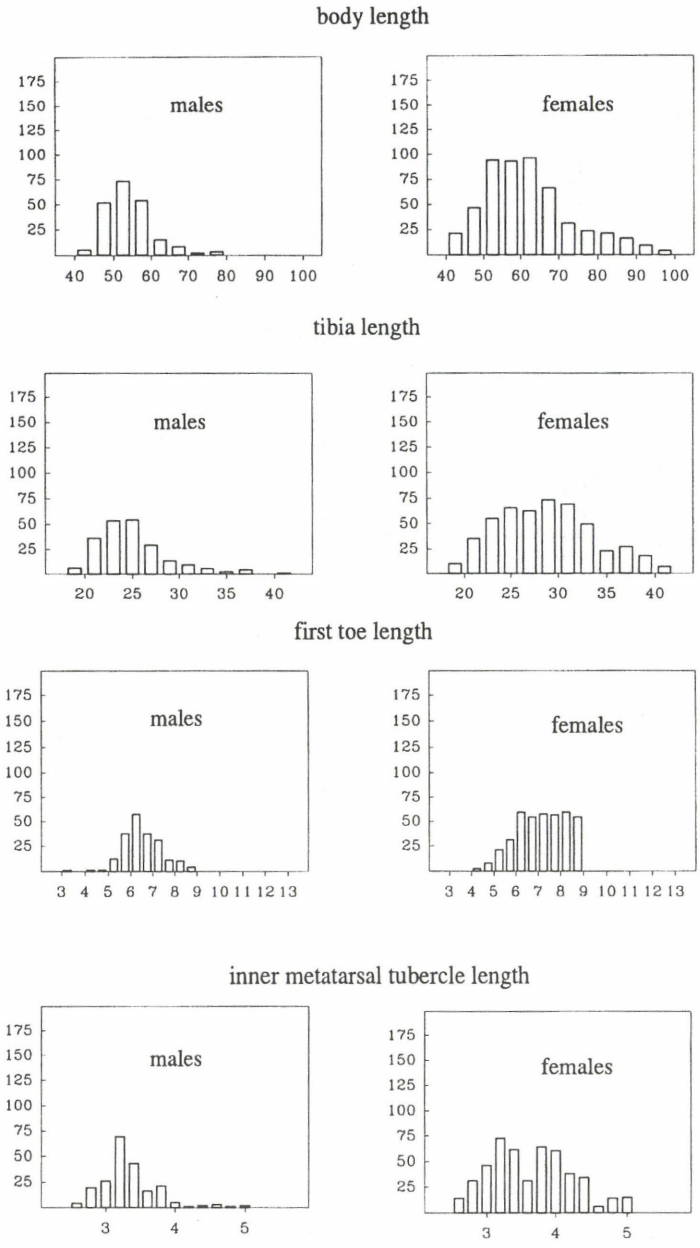

Fig. 2. Frequency distribution of morphometric characters for *Rana esculenta* (horizontalaxes represent the measurements in mm; vertical axes represent the number of specimens)

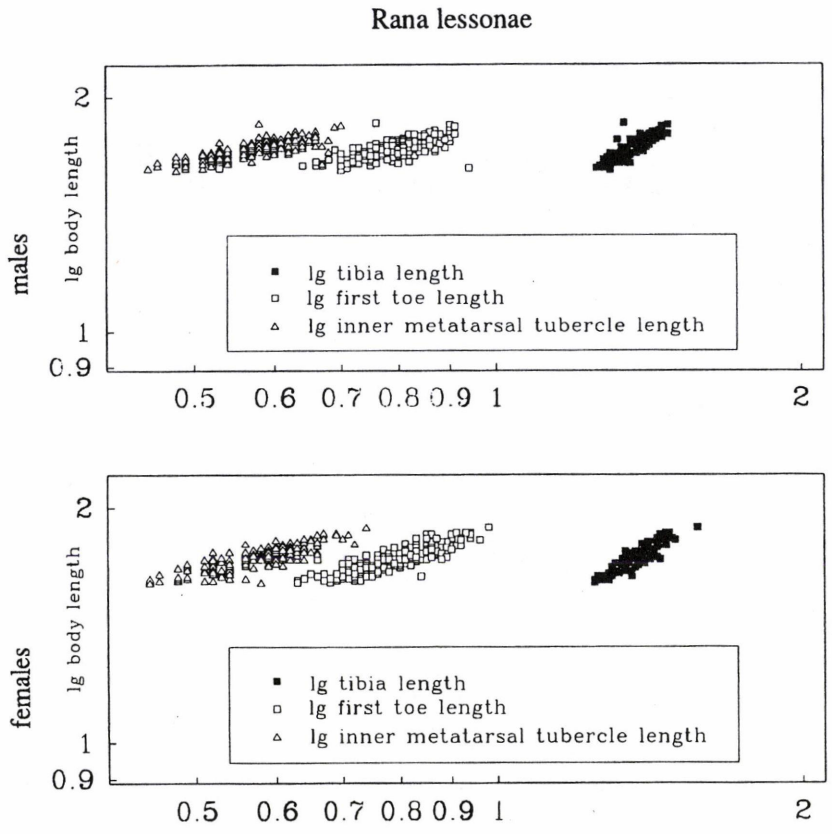

Fig. 3. Allometric growth rate of morphometric characters for *Rana lessonae*

%). On the other hand, misclassifications were rather found among *Rana* kl. *esculenta* specimens. Percentage of frogs correctly classified was 85 %, which seems to be a good result.

The summary of descriptive statistics of 4 morphometric measurements for the two water frog forms are given in Table 3. Means of the measurements for female specimens in both forms exceeded that of males. At the same time, standard deviation of measurements for males proved to be lower than that of the females. A significant sexual dimorphism was found between male and female specimens of pool frog (*Rana lessonae*) in body length, tibia length and first toe length ($P<0.05$). Significant difference between sexes, however, have not been detected in the case of inner metatarsal tubercle length for pool frog ($P = 0.11$). On the other hand, all of the four measurements of *Rana* kl. *esculenta* showed significant differences between the sexes ($P<0.01$). When comparing means of the measurements of the two water frog forms, the value of *Rana* kl. *esculenta* specimens proved to be higher than that of the *Rana lessonae* ones. The differences in measurements were significant ($P<0.01$) between male specimens of the two forms, except for body length ($P = 0.07$). Females of water frog form differed from each other in all of the four morphometric characters ($P<0.02$).

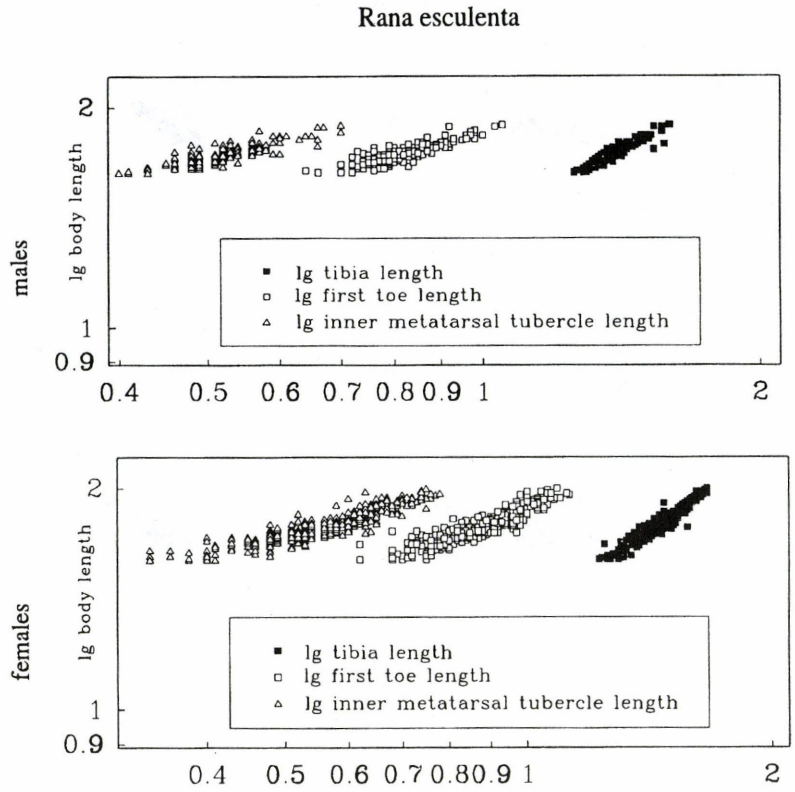

Fig. 4. Allometric growth rate of morphometric characters for *Rana esculenta*

Frequency distributions of the values of body length, tibia length and first toe length approximately followed the desired normal distribution curve in all cases, except the distribution pattern of first toe length for females of *Rana* kl. *esculenta* (Figs 1–2). Frequency distributions of inner metatarsal tubercle length in both species showed, however, zigzag patterns.

On the basis of the multiple regression and determination coefficients, considerable correlations were found both between body length and tibia length and between tibia length and first toe length for the two water frog forms (Table 4). Inner metatarsal tubercle length showed on the one hand, a significant correlation with tibia length, on the other hand a moderate correlation with body length in the cases of *Rana lessonae* specimens. Inner metatarsal tubercle length of *Rana* kl. *esculenta* specimens correlated with both tibia length and body length at similar level.

An allometric growth rate was found between linear body length and the other linear morphometric characters (Figs 3–4). The growth rate of both tibia length and the first toe length compared to body length was similar. Opposite to the above mentioned two measurements, the growth rate of inner metatarsal tubercle was near equal with that of the body length.

When testing the morphometric indices, included into identification function key, sexual dimorphism has not been found using by Mann-Whitney U-test ($P>0.05$). Based on regression and determination coefficients the two indices proved to be independent from each other.

Discussion

Rana lessonae – *Rana* kl. *esculenta* population systems have mostly (99 %) been found in the floodplain of River Danube (Szigetköz). Mean values of morphometric characters of water frog forms, with the exception of male *Rana lessonae*, were lower than the values of frogs collected in other part of Hungary (cf. Gubányi 1992).

Mean values of *Rana lessonae* specimens generally were lower than the data of frogs, collected in the vicinity of Gabcikovo (Lác 1959) situated on the other side of the River Danube. However, maximum values of morphometric characters for *Rana lessonae* specimens were higher in Szigetköz.

As Figs 1-3 showed, distribution pattern of morphometric characters of the two water frog forms represented normal distribution, except inner metatarsal tubercle for both forms and first toe length for female *Rana* kl. *esculenta* specimens. These results were in controversy with the frequency distribution data of body length published by Berger (1966), showing characteristic features of multiple peaked curves. Independence of the two indices, included the identification function, could arise the information bases of the key.

As mentioned in the introduction, according to Wijnands & Gelder (1976) two indices BL/IMTL and BL/IMTW can discriminate the three water frog forms at high level from each other. However, the measurement of IMTW from technical point of view is a bit complicated, thus the indices applied by me seem to be more suitable for field work.

Allometric growth rate, which was detected after transforming the measurements into linear forms, also supported the use of indices, because the growth rate of the investigated morphometric characters cannot represent any symmetry, i.e. they have not isometric growth.

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